

Stem Cell Marker Expression in Mouse Lung after Exposure to Acute Photons and Protons With and Without Pre-exposure to Low-dose Photons

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Purpose: To compare stem cell specific marker expression in mouse lung after whole-body exposure to irradiation with either photons or protons and evaluate modulating effects of pre-irradiation with low-dose/low-dose-rate (LDR) photons on single irradiation alone. Materials and Methods: LDR radiation was delivered to a total dose of 0.01 Gy (0.03 cGy/min, ^{57}Co); additional groups received 2 Gy photons (0.8 Gy/min) or protons (0.9 Gy/min), alone or immediately after LDR. Eighty-four relevant genes were assessed utilizing reverse transcriptase-polymerase chain reaction, and expression and distribution of 6 proteins specific for or associated with lung stem cell differentiation were compared using immunohistochemistry on days 21 and 56 post-irradiation in lung tissue from C57BL/6 mice. Expression of genes with ≥ 2 fold difference and $p < 0.05$ compared to 0 Gy are presented. Results: On day 21, more genes were modulated in response to protons alone and the combination of LDR+protons than the other groups vs 0 Gy or LDR. When compared with 0 Gy, 5 genes (*colla1*, *aldh2*, *bglap1*, *bmp2* and *ppard*) were up-regulated and 2 genes (*col2a1* and *hspa9*) were down-regulated after 2 Gy protons; none were down-regulated by LDR+protons. Among these affected genes, *colla1* was up-regulated by photons, protons and LDR+protons. When protons were compared to LDR, there were 5 up-regulated (*aldh2*, *bmp2*, *colla1*, *foxa2* and *notch1*) and 4 down-regulated (*fgfr1*, *fgf4*, *hspa9* and *rb1*) genes; 7 genes were up-regulated (*bp3*, *btrc*, *foxa2*, *frat1*, *gdf2* and *notch1*) and 1 was down-regulated (*hspa9*) after LDR+protons. On day 56, fewer genes were affected by radiation when compared with 0 Gy than compared with LDR. Comparison with LDR showed 8 up-regulated genes after photons alone, and 9 up-regulated after LDR+protons; one were down-regulated in both groups. Histopathology showed that all radiation regimens recruited inflammatory cells. Immunohistochemistry showed no difference in immunoreactivity among groups for pulmonary stem cell marker prosurfactant protein C (pSP-C), a specific stem cell marker for type II pneumocytes; while immunostaining intensity for another pulmonary stem cell marker, secreted uteroglobin by Clara cells of bronchiolar epithelium, was slightly decreased by both combination of radiations on days 21 and 56. Immunoreactivity for Oct4, a transcription factor expressed by undifferentiated embryonic stem cells, was slightly increased by irradiation with protons alone and the two regimens of combination radiations on day 56. In contrast, immunostaining intensity for slug, involved in epithelial-mesenchymal transition, was slightly decreased after protons and LDR+protons. Unlike gene expression, no marked difference in type I collagen immunostaining intensity was detected among groups. Similarly, immunoreactivity for Grp75 was not changed by the radiations. The Grp75 protein is involved in stress response, intracellular trafficking, antigen processing, control of cell proliferation, differentiation, and tumorigenesis. Conclusions: Taken together, gene expression was affected more profoundly as compared with protein expression. There were no common genes relevant to stem cell markers that were up-/down-regulated by all radiation regimens in this study. Priming with LDR photons prior to acute photon or proton exposure appeared to have little modifying effects on most measured parameters at the times of assessment.

Table 1. Gene expression with ≥ 2 fold difference and $p < 0.05$ compared to 0 Gy on day 21

LDR	2 Gy photons	LDR+2 Gy photons	2 Gy protons	LDR+2 Gy protons
bglap1 2.37	cdc2a (2.28)	col1a1 (2.23)	col1a1 (3.75)	col1a1 (2.01)
			col2a1 (-2.21)	aldh2 (2.00)
			hspa9 (-3.70)	cd4 (2.00)
			aldh2 (2.39)	cdc 2a (2.36)
			bglap1 (2.04)	notch1 (2.12)
			bmp2 (2.06)	
			ppard (2.20)	

Table 2. Gene expression with ≥ 2 fold difference and $p < 0.05$ compared to LDR on day 21

2 Gy photons	LDR+2 Gy photons	2 Gy protons	LDR+2 Gy protons
	foxa2 (2.49)	aldh2 (2.02)	bmp3 (2.19)
	notch1 (2.05)	bmp2 (2.01)	btrc (2.49)
		col1a1 (2.45)	foxa2 (2.62)
		fgfr1 -2.84	frat1 (2.05)
		fgf4 (-2.14)	gdf2 (2.92)
		foxa2 (2.36)	myst2 (2.10)
		hspa9 (-5.59)	notch1 (2.70)
		notch1 (2.47)	hspa9 (-2.26)
		rb1 (-2.10)	

Table 3. Gene expression with ≥ 2 fold difference and $p < 0.05$ compared to 0 Gy on day 56

LDR	2 Gy photons	LDR+2 Gy photons	2 Gy protons	LDR+2 Gy protons
fgfr1 (-2.26)	col9a1 (-3.23)		cdh2 (-2.11)	hspa9 (-4.44)
hspa9 (-4.37)	ascl2 (2.26)		cd19 (2.00)	
t (-2.53)				

Table 4. Gene expression with ≥ 2 fold difference and $p < 0.05$ compared to LDR on day 56

2 Gy photons	LDR+2 Gy photons	2 Gy protons	LDR+2 Gy protons
ccnd1 (2.17)	bglap1 (2.38)	bglap1 (2.41)	abcg2 (2.02)
dhh (2.24)	hspa9 (4.33)	bmp2 (2.01)	actc1 (2.75)
dvl1 (2.44)		cdc2a (2.21)	bmp2 (2.18)
fgf1 (2.18)			dvl1 (2.53)
foxa2 (2.13)			foxa2 (2.09)
gdf2 (2.10)			gdf3 (2.69)
gjb1 (2.14)			gjb1 (2.46)
hspa9 (4.81)			ncam1 (2.33)
			ppard (2.92)

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